

Title: Reproducibility of Acute Steroid Hormone Responses in Men to Short-Duration Running

Submission type: Original investigation.

Authors: Diogo V. Leal^{1,2*}, Lee Taylor^{3,4}, John Hough^{1,5}

Affiliations:¹Institute of Sport and Physical Activity Research, School of Sport and Physical Activity, University of Bedfordshire, Bedford, Bedfordshire, United Kingdom;

²Research Center in Sports Sciences, Health Sciences and Human Development, University Institute of Maia, Maia, Portugal;

³ASPETAR, Qatar Orthopaedic and Sports Medicine Hospital, Athlete Health and Performance Research Centre, Aspire Zone, Doha, Qatar;

⁴School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom;

⁵School of Science and Technology, Nottingham Trent University, Nottingham, NG11 8NS, United Kingdom.

***Address for Correspondence:**

Diogo Vaz Leal
University Institute of Maia, Av. Carlos Oliveira Campos,
4475-690 Castelo da Maia, Portugal

Email: diogo.leal@ismai.pt **Phone:** +351 22 986 60 00

ORCID: 0000-0002-4046-6820

Preferred Running Head: Steroid Reproducibility to Running Bouts

Abstract word count: 250

Text-only word count: 3040

Number of figures: 2

Number of tables: 2

References: 27

40 **Abstract**

41 **Purpose:** Progressively overloading the body to improve
42 physical performance may lead to detrimental states of
43 overreaching/overtraining syndrome (OTS). Exercise-induced
44 cortisol and testosterone have been suggested as overreaching
45 markers with blunted cycle-induced concentrations found
46 following an intensified-training period. To be inclusive for a
47 running population, this study develops two 30-min running
48 bouts: the 50/70 (based on individualized velocity at maximal
49 oxygen uptake) and the RPE_{TP} (self-paced bout) and examines
50 the reproducibility of plasma cortisol and testosterone
51 responses to these bouts. **Methods:** Thirteen recreationally
52 active, healthy males completed each running bout on three
53 occasions, respecting time of day and blood was drawn Pre-,
54 Post- and 30 min Post-Exercise. **Results:** Cortisol did not
55 change in response to 50/70 or RPE_{TP} ($p > 0.05$, $\eta^2 = 0.090$ and
56 $\eta^2 = 0.247$, respectively). Elevated (both $p < 0.01$) testosterone
57 (50/70: 35%, $\eta^2 = 0.790$; RPE_{TP}: 42%, $\eta^2 = 0.876$) was
58 observed, with good intra-individual coefficients of variation
59 (CV_i) as mean \pm standard deviation for cortisol (50/70: $13 \pm$
60 10% ; RPE_{TP}: $12 \pm 7\%$) and testosterone (50/70: $7 \pm 5\%$;
61 RPE_{TP}: $12 \pm 9\%$). Heart rate and rating of perceived exertion
62 were unchanged across trials (all CV_i $< 5\%$, $p < 0.05$).
63 **Conclusions:** Both tests elicited reproducible physiological and
64 hormonal responses. Advantageously for the practitioner,
65 RPE_{TP} does not require *a priori* determination of exercise
66 intensities, unlike the 50/70, enhancing its potential integration
67 into practice. Additionally, RPE_{TP} induces greater disturbances
68 to OTS-implicated hormones compared to 50/70 and may
69 therefore provide a more sensitive tool to highlight
70 NFOR/OTS.

71 **Keywords:** Performance, running test, stress, overreaching,
72 prevention.

73

74

Introduction

Successful athletic training requires balanced overload and recovery, without which short-term performance decrements can occur (e.g. overreaching) in as little as 7 days.¹ Importantly, whilst overreached athletes can experience performance decrements in the short-term, sufficient recovery (days to weeks) facilitates a “supercompensatory” performance enhancing effect [e.g. functional overreaching (FOR)²]³. Without sufficient recovery from periods of overload, “non-functional overreaching” (NFOR) can occur (requiring weeks/months to recover from fully) with NFOR complicit in the more protracted overtraining syndrome (OTS; requiring several months or even years to recover from fully).²

Resting concentrations of cortisol and testosterone were suggested as markers of overreaching/NFOR/OTS yet their efficacy in these regards is inconclusive with increases, decreases and no changes in concentrations under examination before to after intensified-training periods.⁴⁻⁶ Exercise-induced responses appear to have greater utility, with blunted ACTH and cortisol responses to 2 consecutive continual incremental cycles to fatigue identified following a 10-day intensified-training period, compared with pre-training.⁷ Following on from these findings, robust elevations of salivary cortisol (~120%) and testosterone (~33%) to a continuous, 30-min cycle bout, consisting of alternating blocks of 1 min at 55% maximal workload (\dot{W}_{\max}) and 4 min at 80% \dot{W}_{\max} (i.e. the 55/80) were reported,⁸ with blunted exercise-induced salivary cortisol and testosterone in response to the 55/80 shown following an 11-day⁹ and salivary testosterone after a 10-day¹⁰ intensified-training period.

However, despite some utility for the 55/80 to highlight exercise-induced overreaching-related hormonal imbalances in cyclists, its application within other athletes (e.g. runners) is evidently lacking. Given a 30-min running bout at 80% of maximal oxygen uptake ($\dot{V}O_{2\max}$) has been reported to elevate plasma cortisol by ~20%,¹¹ and a running test to exhaustion at 100% ventilatory threshold increased plasma cortisol (~97%) and total testosterone (31%),¹² it was hypothesized that a short duration running protocol variant of the cycling 55/80 may be viable. This running variant, theoretically, could induce an acute elevation in plasma cortisol and testosterone when in a healthy state and also detect alterations in the exercise-induced responses of these hormones as a consequence of intensified-training period. To be of value in practice, this variant protocol must demonstrate reproducible hormone and physiological responses when participants are in a rested healthy state.

125 The aim of this study is to therefore examine whether the acute
126 plasma cortisol and testosterone responses to two novel,
127 continuous, 30-min treadmill-run protocols are reproducible,
128 within rested yet active healthy participants, aiming to design a
129 short-duration running bout that could be practically used to
130 prevent the incidence of NFOR/OTS.

131

132 **Methods**

133

134 **Subjects**

135

136 In a randomized crossover design, 13 recreationally active
137 males¹³ volunteered to participate (Table 1). This study was
138 granted ethical approval by the University of Bedfordshire
139 Research Ethics Committee (2014ISPAR003) in accordance
140 with the 2013 Declaration of Helsinki. After comprehensive
141 verbal and written descriptions of the study, written informed
142 consent was provided by participants.

143

144 **(*** Insert Table 1 near here ***)**

145

146 **Design**

147

148 On the first visit to the laboratories a submaximal and a
149 $\dot{V}O_{2\max}$ tests were completed on a motorised treadmill (PPS55
150 Med-i, Woodway, Weil am Rhein, Germany). On the following
151 visits, 7 separate trials were completed – 6 main experimental
152 trials and one control, resting trial (CTL). All trials were
153 completed at 12:00 to avoid the influence of diurnal variation
154 of the hormones being examined (Figure 1). To avoid baseline
155 peak circulating cortisol levels due to circadian rhythm, all
156 participants were asked to wake up no later than 08:00 on the
157 morning of the trial. A standard breakfast chosen by the
158 participant was consumed before 09:00 and was replicated
159 before each main trial. Participants were requested to drink
160 ~500 mL of water in the morning of the trial and euhydration
161 was confirmed by a urine osmolality of $\leq 700 \text{ mOsm}\cdot\text{kg}\cdot\text{H}_2\text{O}^{-1}$.¹⁴
162 All participants reported to the laboratory at ~11:30 and
163 completed a 76-statement recovery-stress questionnaire
164 (RESTQ-76). The RESTQ-76 discriminates 48 nonspecific and
165 28 sport-specific areas of stress and recovery, consisting of 19
166 main scales in total.¹⁵ Each of these subscales includes specific
167 statements. The sum of scores (answer to each statement) in
168 each of the subscales is used to examine the overall responses
169 to the questionnaire. Each answer ranges from never (0) to
170 always (6) and covers the participants' past 3 days. Participants
171 did not consume any food until the end of each main
172 experimental trial but were allowed to drink water *ad libitum*
173 throughout the exercise bouts. Body mass was measured pre-
174 and post-exercise and heart rate (HR) and rating of perceived

175 exertion (RPE) were measured in the last 15 s of each stage
176 during the exercise bouts via short-range radio telemetry (Polar
177 FT1, Polar Electro Oy, Kempele, Finland) and the 6-20 Borg
178 scale, respectively.

179
180 A similar diet was consumed during the 24 hours preceding
181 each trial and measured via a weighed food diary. A nutrition
182 analysis software (Dietplan, Version 6.70.74, Forestfield, West
183 Sussex, UK) was used to determine mean energy (9439 ± 3954
184 kJ), carbohydrate ($58\% \pm 12\%$), fat ($27\% \pm 13\%$), and protein
185 ($14\% \pm 2\%$) intake.

186 187 **Methodology**

188
189 A 3-min warm-up run at $7 \text{ km}\cdot\text{h}^{-1}$ and 1% gradient was
190 undertaken prior to the submaximal test. A 4-stage, 16-min,
191 incremental treadmill-run test was then completed in order to
192 determine the running speed/oxygen consumption ($\dot{V}\text{O}_2$)
193 relationship.¹⁶ The initial speed was self-selected between $6.5 -$
194 $12.0 \text{ km}\cdot\text{h}^{-1}$. Speed was then increased by $1 \text{ km}\cdot\text{h}^{-1}$ every stage.
195 A 20-min resting recovery was then undertaken. $\dot{V}\text{O}_{2\text{max}}$ was
196 assessed using an incremental incline-ramped test.¹⁶ The
197 gradient was increased by 1% every minute until volitional
198 exhaustion. The initial speed was set at the speed corresponding
199 to a HR of $\sim 150 \text{ beats}\cdot\text{min}^{-1}$ (range: $9.5 - 13.0 \text{ km}\cdot\text{h}^{-1}$) on the
200 submaximal test and remained constant throughout. Expired
201 gas was analysed by using a breath-by-breath ergospirometry
202 system (MetaLyzer 3B, Cortex, Leipzig, Germany). The
203 $v\dot{V}\text{O}_{2\text{max}}$ was determined by regressing $\dot{V}\text{O}_2$ exercise intensity
204 for submaximal exercise and extrapolating this relationship to
205 $\dot{V}\text{O}_{2\text{max}}$.¹⁷

206
207 **(*** Insert Figure 1 near here ***)**

208
209 In the 6 main exercise trials the participants completed each of
210 the 2 designed running bouts on 3 separate occasions - 1
211 familiarisation (FAM) and 2 main trials (T1 and T2), to avoid
212 any learning effects. All trials were randomly assigned.
213 Participants abstained from exercise, caffeine and alcohol
214 intake 24 hours before each main trial. Blood samples were
215 drawn Pre-, Post-, and 30 min Post-Exercise in T1 and T2. The
216 tests were both 30-min, continuous treadmill-running and were
217 designed as follows: (a) alternating blocks of 1 min at 50%
218 $v\dot{V}\text{O}_{2\text{max}}$ and 4 min at 70% $v\dot{V}\text{O}_{2\text{max}}$ (50/70); (b) alternating 1
219 min at an RPE of 11 (fairly light) and 4 min at 15 (hard) on the
220 6-20 Borg scale (RPE_{TP}), where the treadmill speed could be
221 adjusted but not seen by the participant to maintain the RPE in
222 the target range; (c) a 30-min no exercise, control trial (CTL)
223 (Figure 1). In all exercise trials, the treadmill slope was set at
224 1% gradient.

225 *Analytical Procedures:* Whole blood samples were collected by
 226 venepuncture from an antecubital vein into 5 mL tri-potassium
 227 ethylenediaminetetraacetic acid (K₃EDTA) vacutainers
 228 (Vacuette, Greiner Bio-One, Stonehouse, UK). Blood was
 229 centrifuged at 1500 g for 10 min at 4°C (Heraeus Multifuge
 230 X3R, Thermo Scientific, Loughborough, UK) and plasma was
 231 transferred into 1.5 mL aliquots (Eppendorf, Hamburg,
 232 Germany) to be stored at -80°C. Plasma cortisol and
 233 testosterone concentrations were determined by using
 234 commercially available enzyme-linked immunosorbent assay
 235 (ELISA) kits (IBL International, Hamburg, Germany). All
 236 samples were analysed in duplicate and average concentrations
 237 were used. The sensitivity of the plasma cortisol and
 238 testosterone kits is 6.8 nmol.L⁻¹ and 0.29 nmol.L⁻¹, respectively
 239 and the mean intra-assay CV were 3.0% (cortisol) and 4.6%
 240 (testosterone), according to the manufacturers specifications.
 241 The mean inter-assay CV were 3.5% and 5.7% for cortisol and
 242 testosterone, respectively.

243

244 **Statistical Analysis**

245 Statistical analyses were accomplished by using the IBM
 246 Statistical Package for Social Sciences® (SPSS) Statistics
 247 version 23.0 (SPSS Inc., Chicago, IL). Raw data were checked
 248 for normality and homoscedasticity, using the Shapiro-Wilk
 249 test and scatter plots, respectively. Non-normally distributed
 250 data sets were log transformed (to base 10) and rechecked for
 251 normality. Normally distributed data sets (plasma cortisol and
 252 testosterone) were analysed using a two-way repeated measures
 253 analysis of variance (ANOVA). On finding an effect, paired
 254 sample t-tests were used with Bonferroni adjustments. Partial
 255 eta squared (η^2) values were used to examine the size of the
 256 effect when examining the exercise-induced response of plasma
 257 cortisol and testosterone. A one-way repeated measures
 258 ANOVA with paired-sample t-test with Bonferroni corrections
 259 was used to examine HR and speed in CTL and exercise trials,
 260 and hormonal responses during CTL. Reproducibility analysis
 261 was accomplished by determining the CV_i of all physiological
 262 and hormonal measurements. The CV_i were presented as a
 263 percentage and were calculated by hand using the equation CV_i
 264 $= (SD_t/\bar{X}_t)*100$, where SD_t is the standard deviation of the
 265 hormone responses to the main experimental trials averaged,
 266 and \bar{X}_t is the average of the hormone concentrations at Pre-,
 267 Post- and 30 min Post-Exercise averaged¹⁸. The ICC used was
 268 a two-way model, based on the examination of single measures,
 269 i.e. ICC (2,1). Cohen's *d* effect sizes (ES) were used to
 270 examine the magnitude of hormonal change between trials,¹⁹
 271 were calculated by hand as detailed in Vincent and Weir,²⁰ and
 272 were categorized using standardized thresholds of < 0.2 trivial,
 273 0.21 – 0.60 small, 0.61 – 1.20 moderate, 1.21 – 2.0 large, and >
 274 2.0 very large.¹⁹ The alpha level of significance was set as $p <$

0.05. Data is reported as mean \pm SD. All results were presented as raw data to facilitate its comprehension.

Results

Hydration status: Urine osmolality did not differ across all trials and was 348 ± 204 mOsmol \cdot kg⁻¹ H₂O in T1, 351 ± 200 mOsmol \cdot kg⁻¹ in T2 (50/70), 345 ± 198 mOsmol \cdot kg⁻¹ H₂O in T1, 310 ± 168 mOsmol \cdot kg⁻¹ in T2 (RPE_{TP}) and 301 ± 166 mOsmol \cdot kg⁻¹ H₂O in CTL ($p > 0.05$).

Recovery-Stress Questionnaires: No changes in the RESTQ-76 Sport scores were found in any of the stress or recovery scales across all trials ($p > 0.05$).

Physiological Responses to Exercise: No differences in HR or speed were found when comparing FAM, T1 and T2 in any of the exercise bouts ($p < 0.05$). When comparing both exercise bouts, a significant trial effect for speed, HR and RPE was found ($p < 0.01$). Average speed and HR were 21% and 9% higher in the RPE_{TP} compared with the 50/70, respectively. The RPE scores in the RPE_{TP} were ~17% higher than in the 50/70. Reproducibility data for speed, HR and RPE and average HR and speed in response to the 50/70 and RPE_{TP} are presented in Table 2.

(*** Insert Figure 2 near here ***)

Hormonal Responses During CTL: Plasma cortisol decreased from Pre- to Post-CTL ($p < 0.01$) by $\sim 18\% \pm 16\%$. Plasma testosterone did not alter over time ($p > 0.05$ for all).

Hormonal Responses to Exercise: No trial effect was observed in the 50/70 ($p = 0.65$) or the RPE_{TP} ($p = 0.72$) when examining plasma cortisol responses. A time effect was observed in the 50/70, with cortisol decreasing from Post-Exercise to 30-min Post-Exercise ($p < 0.01$, $\eta^2 = 0.090$). No time effect was found in the RPE_{TP} ($p = 0.07$, $\eta^2 = 0.247$). Cortisol levels changed from Pre- to Peak Post-Exercise by -3% and +29% (50/70), and by +34% and +47% (RPE_{TP}) in T1 and T2, respectively. Individual exercise-induced changes are presented in Figure 2. Pre-Exercise cortisol samples did not differ ($p = 0.89$) across trials. No trial effect was observed when comparing the 50/70 with the RPE_{TP} ($p = 0.35$). For plasma testosterone, no trial effect was found when comparing T1 and T2 in the 50/70 ($p = 0.51$) and the RPE_{TP} ($p = 0.49$). However, a significant time effect was shown in 50/70 ($p < 0.001$) and the RPE_{TP} ($p < 0.001$). Pairwise comparisons showed testosterone acutely elevated in all exercise trials and remained elevated at 30 min Post-Exercise in the RPE_{TP} (both $p < 0.01$, $\eta^2 = 0.790$ and $\eta^2 =$

0.876 in the 50/70 and RPE_{TP}, respectively). Testosterone levels changed from Pre- to Post-Exercise by +30% and +39% (50/70), and by +46% and +38% (RPE_{TP}) in T1 and T2, respectively. Individual exercise-induced changes are presented in Figure 2. Pre-Exercise testosterone samples did not differ ($p = 0.66$) across trials. No trial effect was observed when comparing the 50/70 with the RPE_{TP} ($p = 0.11$). All reproducibility data and average plasma cortisol and testosterone concentrations for T1 and T2 are presented in Table 2.

(*** Insert Table 2 near here ***)

Discussion

This study aimed to examine the responses of plasma cortisol and testosterone responses to 2 different continuous, 30-min, high-intensity running bouts and the reproducibility of these responses. It was hypothesized that the hormonal concentrations would acutely elevate in response to all bouts and that these responses would be reproducible. The intra-individual variability in plasma cortisol and testosterone observed in this present study are within the normal variability associated with these hormones, and therefore support the reproducibility of the hormonal responses to the 50/70 and the RPE_{TP}. In fact, the RPE_{TP} (a potentially more practically applied field test due to its self-paced design) has shown to elicit greater physiological responses than the 50/70 bout, as well as reproducible plasma cortisol and testosterone responses. However, only plasma testosterone markedly elevated in response to this running tool, suggesting testosterone may be a better indicator of an exercise-related stress reaction.

Cortisol is known to be a stress-related hormone that rises during and after psychological stress.²¹ Analysis of the scores to the RESTQ-76 showed no disparities in any of the scales, detailing the participants were in a similar state of predisposition to undertake physical activity on every trial and therefore the hormonal responses reported have not been influenced by a change in wellbeing.

The reproducibility of the physiological responses to both tests was examined. Being a self-paced tool, the RPE_{TP} could provoke different HR responses if the speeds chosen by the participants were different when completing the bouts on different occasions. In this study, HR and speed did not alter across all exercise trials. These results are important, as an alteration in the speeds would be indicative of a subsequent alteration in exercise intensity, and therefore influence the response of both cortisol and testosterone. Additionally, the HR

375 and speed responses were shown to be reproducible to both
376 tests with CV_i of $2.9 \pm 2.1\%$ for HR (50/70), and $1.8 \pm 1.3\%$
377 and $2.2 \pm 1.8\%$ for HR and speed (RPE_{TP}). These data suggest
378 that both bouts induced a similar physiological strain, hence the
379 similar HR, RPE and running speeds.

380

381 Similar studies to this one have reported a significant elevation
382 of salivary cortisol and testosterone in response to a continuous
383 30-min, cycle bout when in a healthy state.⁸⁻¹⁰ Duration and
384 intensity of exercise sessions are two important factors known
385 to cause an exercise-induced increase in plasma and salivary
386 cortisol concentrations,²² with exercise intensity above 60%
387 $\dot{V}O_{2max}$ for at least 20-30 min being required for cortisol to
388 elevate.²³ In this current study, plasma cortisol did not
389 significantly increase to either the 50/70 or the RPE_{TP}. There
390 was, however, a percentage-elevation from Pre- to Post-
391 Exercise in both trials in the RPE_{TP} (34% and 47%) and in T2
392 in the 50/70 (29%). Individual cortisol levels show contrasting
393 responses, ranging from moderate decreases to robust
394 increases. As the RPE_{TP} is a self-paced bout, each participant
395 exercised at an intensity dependant of an individual perceived
396 exertion. Although the RPE_{TP} bout was designed to elicit an
397 RPE of 15 (hard) for the majority of the test (24 min), it was
398 not confirmed whether this would provoke an exercise intensity
399 stressful enough to acutely elevate cortisol levels. However, a
400 consistent exercise-induced elevation in plasma testosterone
401 was seen in all exercise trials. Furthermore, testosterone levels
402 did not change with time during CTL, whereas cortisol
403 significantly decreased from Pre- to Post-CTL. It may be
404 reasonable to suggest that the circadian rhythm of cortisol is
405 likely to have led to 50/70 and RPE_{TP} being unable to induce
406 the hypothesised acute elevation, which was not assumed due
407 to Hough *et al.*⁸ reporting no alteration in resting plasma
408 cortisol between 12:00-13:00. Cortisol is known to have a high
409 intra-individual variability.²⁴ When examining the intra-
410 individual variation across trials this study shows an intra-
411 individual variation of ~13% and ~12% in plasma cortisol in
412 the 50/70 and RPE_{TP}, respectively. At first examination, these
413 data may seem a little high, however, the within-subject
414 variability in cortisol has been reported to be ~21.7%.²⁵ The
415 CV_i for testosterone is also within the 12.6%²⁵ and the 11.8%²⁶
416 intra-individual variability, suggesting the variability found
417 falls within normal biological variability values reported
418 previously. Any shift from the reported variation may be due to
419 the fact these studies have examined the variability of resting
420 levels, while the present study has looked at the exercise-
421 induced responses. ES were used to examine the magnitude of
422 change between trials, with Cohen²⁷ proposing that small
423 differences would be described if presenting an ES value of
424 0.21. The ES for cortisol and testosterone were 0.07 and 0.04

425 (50/70) and 0.03 and 0.04 (RPE_{TP}), respectively. These data
426 support the trivial changes in the hormones examined in this
427 study when compared across trials.

428

429 **Practical applications**

430

- 431 • Testosterone may be a better indicator of a hypothalamic-
432 pituitary activation following short-duration, high-intensity
433 exercise when compared to cortisol.
- 434 • Both tests elicited reproducible plasma cortisol responses
435 but did not acutely elevate its concentration. This means it
436 may be inappropriate to measure cortisol as a biomarker to
437 highlight exercise-induced stress.
- 438 • Testosterone elevated in both tests and these responses were
439 reproducible. The intra-individual variability of testosterone
440 responses is at a level that suggests that both tests could
441 highlight blunted acute responses following an intensified-
442 training period, emphasising its usefulness to prevent and
443 avoid the incidence of NFOR/OTS.
- 444 • The RPE_{TP} is a self-paced running bout, hence it does not
445 require preliminary testing for determination of exercise
446 intensities. Therefore, it may be more practically applied in
447 an athletic/elite population and its short duration may be
448 advantageous if incorporating it within a training session.

449

450 **Conclusions**

451

452 Hypothetically cortisol and testosterone would acutely elevate
453 in response to both tests and these would provoke reproducible
454 hormonal and physiological responses. We propose that cortisol
455 is very individualised, and the exercise-induced responses may
456 be influenced by a circadian rhythm. Additionally, using the
457 RPE_{TP} may be more practically applied in the field as it will not
458 require preliminary testing to determine exercise intensities.

459

460

461 **Acknowledgements**

462 The authors would like to acknowledge all participants
463 involved in this study and Mr William Craggs for his help with
464 data collection and recruitment of participants, and the
465 technical staff at the laboratory for their support. The authors
466 have no conflict of interest to report.

467

468

469

470

471

472

473

474

475 **References**

- 476 1. Halson SL, Bridge MW, Meeusen R, et al. Time course
477 of performance changes and fatigue markers during
478 intensified training in trained cyclists. *J Appl Physiol.*
479 2002;93(3):947-956.
480 doi:10.1152/jappphysiol.01164.2001

- 481 2. Meeusen R, Duclos M, Foster C, et al. Prevention,
482 diagnosis and treatment of the overtraining syndrome:
483 Joint consensus statement of the European College of
484 Sport Science (ECSS) and the American College of
485 Sports Medicine (ACSM). *Eur J Sport Sci.*
486 2013;13(1):1-24. doi:10.1080/17461391.2012.730061

- 487 3. Halson SL, Jeukendrup AE. Does overtraining exist? An
488 analysis of overreaching and overtraining research. *Sport*
489 *Med.* 2004;34(14):967-981. doi:10.2165/00007256-
490 200434140-00003

- 491 4. Hoogeveen a R, Zonderland ML. Relationships between
492 testosterone, cortisol and performance in professional
493 cyclists. *Int J Sports Med.* 1996;17(6):423-428.
494 doi:10.1055/s-2007-972872

- 495 5. Lucia A, Diaz B, Hoyos J, et al. Hormone levels of
496 world class cyclists during the tour of Spain stage race.
497 *Br J Sports Med.* 2001;35(6):424-430.
498 doi:10.1136/bjsm.35.6.424

- 499 6. Grandys M, Majerczak J, Duda K, Zapart-Bukowska J,
500 Kulpa J, Zoladz JA. Endurance training of moderate
501 intensity increases testosterone concentration in young,
502 healthy men. *Int J Sports Med.* 2009;30(7):489-495.
503 doi:10.1055/s-0029-1202340

- 504 7. Meeusen R, Nederhof E, Buyse L, Roelands B, De
505 Schutter G, Piacentini MF. Diagnosing overtraining in
506 athletes using the twobout exercise protocol. *Br J Sports*
507 *Med.* 2010;44(9):642-648.
508 doi:10.1136/bjsm.2008.049981

- 509 8. Hough JP, Papacosta E, Wraith E, Gleeson M. Plasma
510 and salivary steroid hormone responses of men to high-
511 intensity cycling and resistance exercise. *J Strength*
512 *Cond Res.* 2011;25(1):23-31.
513 doi:10.1519/JSC.0b013e3181fef8e7

- 514 9. Hough J, Corney R, Kouris A, Gleeson M. Salivary
515 cortisol and testosterone responses to high-intensity
516 cycling before and after an 11-day intensified training
517 period. *J Sports Sci.* 2013;31(14):1614-1623.
518 doi:10.1080/02640414.2013.792952

- 519 10. Hough J, Robertson C, Gleeson M. Blunting of exercise-
520 induced salivary testosterone in elite-level triathletes
521 with a 10-day training camp. *Int J Sports Physiol*
522 *Perform.* 2015;10(7):935-938. doi:10.1123/ijsp.2014-
523 0360
- 524 11. Verde T, Thomas S, Shephard RJ. Potential markers of
525 heavy training in highly trained distance runners. *Br J*
526 *Sports Med.* 1992;26(3):167-175.
527 doi:10.1136/bjism.26.3.167
- 528 12. Daly W, Seegers CA, Rubin DA, Dobridge JD, Hackney
529 AC. Relationship between stress hormones and
530 testosterone with prolonged endurance exercise. *Eur J*
531 *Appl Physiol.* 2005;93(4):375-380. doi:10.1007/s00421-
532 004-1223-1
- 533 13. de Pauw K, Roelands B, Cheung SS, de Geus B,
534 Rietjens G, Meeusen R. Guidelines to Classify Subject
535 Groups in Sport- Science Research Guidelines. *Int J*
536 *Sports Physiol Perform.* 2013;8(2):111-122.
537 doi:10.1123/ijsp.8.2.111
- 538 14. Sawka MN, Burke LM, Eichner ER, Maughan RJ,
539 Montain SJ, Stachenfeld NS. American College of
540 Sports Medicine position statement: Exercise and Fluid
541 Replacement. *Med Sci Sports Exerc.* 2007;39(2):377-
542 390. doi:10.1249/mss.0b013e31802ca597
- 543 15. Kellmann M, Kallus KW. Recovery-Stress
544 Questionnaire for Athletes. In: *The Recovery-Stress*
545 *Questionnaires.* ; 2016:86-134.
- 546 16. Deighton K, Zahra JC, Stensel DJ. Appetite, energy
547 intake and resting metabolic responses to 60min
548 treadmill running performed in a fasted versus a
549 postprandial state. *Appetite.* 2012;58(3):946–954.
550 doi:10.1016/j.appet.2012.02.041
- 551 17. Jones AM, Vanhatalo AT, Doust JH. From Severe to
552 Extreme Exercise: VO2max and exercise economy. In:
553 Eston R, Reilly T, eds. *Kinanthropometry and Exercise*
554 *Physiology Laboratory Manual: Tests, Procedures and*
555 *Data. Volume 2: Physiology.* 3rd ed. Oxford: Routledge;
556 2009.
- 557 18. Sale D. Testing strength and power. In: MacDougall J.
558 D., Wenger HA, Green HJ, eds. *Physiological Testing of*
559 *the High-Performance Athlete.* Champaign, IL: Human
560 Kinetics; 1991:21-106.
- 561 19. Hopkins WG, Marshall SW, Batterham AM, Hanin J.
562 Progressive statistics for studies in sports medicine and

- 563 exercise science. *Med Sci Sports Exerc.* 2009;41(1):3.
564 doi:10.1249/MSS.0b013e31818cb278
- 565 20. Vincent W, Weir J. *Statistics in Kinesiology*. 4th ed.
566 Champaign, IL: Human Kinetics; 2012.
- 567 21. Burke HM, Davis MC, Otte C, Mohr DC. Depression
568 and cortisol responses to psychological stress: A meta-
569 analysis. *Psychoneuroendocrinology*. 2005;30(9):846-
570 856. doi:10.1016/j.psyneuen.2005.02.010
- 571 22. Kuoppasalmi K, Näveri H, Härkönen M, Adlercreutz H.
572 Plasma cortisol, androstenedione, testosterone and
573 luteinizing hormone in running exercise of different
574 intensities. *Scand J Clin Lab Invest.* 1980;40(5):403-409.
575 doi:10.3109/00365518009101862
- 576 23. Davies C, Few J. Effects of exercise on adrenocortical
577 function. *J Appl Physiol.* 1973;35(6):887-891.
- 578 24. Almeida DM, Piazza JR, Stawski RS. Interindividual
579 Differences and Intraindividual Variability in the
580 Cortisol Awakening Response: An Examination of Age
581 and Gender. *Psychol Aging.* 2009;24(4):819.
582 doi:10.1037/a0017910
- 583 25. Maes M, Mommen K, Hendrickx D, et al. Components
584 of biological variation, including seasonality, in blood
585 concentrations of TSH, TT3, FT4, PRL, cortisol and
586 testosterone in healthy volunteers. *Clin Endocrinol*
587 *(Oxf)*. 1997;46(5):587-598. doi:10.1046/j.1365-
588 2265.1997.1881002.x
- 589 26. Sartorius G, Spasevska S, Idan A, et al. Serum
590 testosterone, dihydrotestosterone and estradiol
591 concentrations in older men self-reporting very good
592 health: The healthy man study. *Clin Endocrinol (Oxf)*.
593 2012;77(5):755-763. doi:10.1111/j.1365-
594 2265.2012.04432.x
- 595 27. Cohen J. *Statistical Power Analysis for the Behavioral*
596 *Sciences*. 2nd ed. Lawrence Erlbaum Associates; 1988.
597 doi:10.1234/12345678

598